Corporation obtaining approval, the name of its representative, and the address of its main office

Applicant:
Name: Syngenta Japan K.K.
Okifumi Murata, CEO
Address: Office Tower X, 1-8-10, Harumi, Chuo-ku, Tokyo

Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Maize resistant to Lepidoptera and Coleoptera, and tolerant to glufosinate herbicide and glyphosate herbicide (modified *cry1Ab*, *cry34Ab1*, *cry35Ab1*, modified *cry3Aa2*, *cry1F*, *pat*, *mEPSPS*, *Zea mays* subsp. *mays* (L.) Iltis) (Bt11×*B.t.* Cry34/35Ab1 Event DAS-59122-7×MIR604×*B.t.* Cry1F maize line 1507×GA21, OECD UI : SYN-BTØ11-1×DAS-59122-7×SYN-IR6Ø4-5×DAS-Ø15Ø7-1×MON-ØØØ21-9) [including the progeny lines isolated from the maize lines, Bt11, *B.t.* Cry34/35Ab1 Event DAS-59122-7, MIR604, *B.t.* Cry1F maize line 1507 and GA21, that contains a combination of any of the transferred genes in the individual maize lines (except those already granted an approval regarding Type 1 Use Regulation)]
Content of the Type 1 Use of Living Modified Organism	Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them
Method of the Type 1 Use of Living Modified Organism	—

Outline of the Biological Diversity Risk Assessment Report

I. Information collected prior to assessing Adverse Effect on Biological Diversity

1. Information concerning preparation of living modified organisms

This stack maize line possesses resistance to Lepidoptera and Coleoptera, and tolerance to glufosinate herbicide and glyphosate herbicide which are derived from five (5) recombinant maize parent lines. In addition, this stack maize line will be commercialized as a hybrid variety (F1) and the grain harvested from this stack maize line is composed of combinations of the transferred genes in the individual parent lines of this stack maize line due to the genetic segregation. Brief information concerning preparation of Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21 are explained individually in the following sections.

(1) Information concerning donor nucleic acid

1) Composition and origins of component elements

The composition of donor nucleic acid and the origins of component elements used for the development of Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21 are shown individually in Table 1 to Table 5 (pp. 2 - 7).

Table 1 Origins and functions of the component elements of the donor nucleic acid used for the development of Bt11

Gene cassette resistant to Lepidoptera	
Component elements	Origin and function
35S promoter	A promoter obtained as *Dde*I-*Dde*I fragment derived from cauliflower mosaic virus (CaMV) CM1841 strain. This promoter makes the target gene (modified *cry1Ab*) expressed in all the tissues constitutively (Reference 14).
IVS6-ADH1	An intron derived from the alcohol dehydrogenase 1S (Adh1-S) gene of maize (Reference 15). Adh1-S intron was used to enhance the expression of target genes (modified *cry1Ab*) in plants (Reference 16).
Modified *cry1Ab*	A modified version of the full-length *cry1Ab* gene that encodes the Cry1Ab protein of *Bacillus thuringiensis* subsp. *kurstaki* HD-1 strain, by partially deleting the C-terminal code region which is independent from the insecticidal activity of the Cry1Ab protein and modifying some nucleotide sequences to change the contents of GC and enhance its expression level in plants. This modification does not change any amino acid sequences of the core protein of the Cry1Ab protein.
NOS term	The 3' untranslated region of nopaline synthase (NOS) gene of *Agrobacterium tumefaciens*, which contains a transcription terminator and a signal for polyadenylation of mRNA (Reference 17, Reference 18). This sequence terminates transcription of target genes (modified *cry1Ab*).

Gene cassettes tolerant to glufosinate herbicide	
Component elements	Origin and function
35S promoter	A promoter obtained as *Alu*I-*Dde*I fragment derived from cauliflower mosaic virus (CaMV) Cabb-s strain. This promoter makes the target gene (*pat*) expressed in all the tissues constitutively (Reference 19).
IVS2-ADH1	An intron derived from the alcohol dehydrogenase 1S (Adh1-S) gene of maize (Reference 15). Adh1-S intron was used to enhance the expression of the target gene (*pat*) in plants (Reference 16).
pat	The gene that encodes the PAT protein of *Streptomyces viridochromogenes*. The PAT protein, that confers glufosinate herbicide tolerance, was used as a selective marker for modified plants at the time of transferring of genes. The *pat* gene has some nucleotide sequences modified to change the GC contents and enhance its expression level in plants. The amino acid sequence of the PAT protein expressed by the modification remains unchanged (Reference 20).
NOS term	The 3' untranslated region of nopaline synthase (NOS) gene of *A. tumefaciens*, which contains a transcription terminator and a signal for polyadenylation of mRNA (Reference 17, Reference 18). This sequence terminates transcription of the target genes (*pat*).
Other regions (hereinafter referred to as "Backbone region")	
Component elements	Origin and function
ColE1 ori	The replication origin derived from *Escherichia coli* plasmid pUC18 (Reference 21, Reference 22). Permits replication of plasmid in bacteria.
amp^R	Derived from *Escherichia coli* (*E. coli*), it has the function to code β-lactamase and confer the tolerance to antibiotic ampicillin (Reference 22).

5

Table 2 Origins and functions of the component elements of the donor nucleic acid used for the development of Event DAS-59122-7

cry34Ab1 gene expression cassette	
Component elements	Origin and function
UBI1ZM PRO	Ubiquitin constitutive promoter derived from *Zea mays* [1)] (including intron and 5' untranslated region)
cry34Ab1	A gene that encodes Cry34Ab1 protein derived from *B. thuringiensis* PS149B1 strain
PIN II *TERM*	Protease inhibitor II terminator to terminate transcription derived from *Solanum tuberosum* (including intron and 5' untranslated region)

cry35Ab1 gene expression cassette	
TA Peroxidase PRO	Peroxidase promoter (nucleotide sequence 45-1342 of GenBank X53675) derived from *Triticum aestivum* known to express in roots
cry35Ab1	A gene that encodes Cry35Ab1 protein derived from *B. thuringiensis* PS149B1 strain
PIN II *TERM*	Protease inhibitor II terminator to terminate transcription derived from *S. tuberosum* (including intron and 5' untranslated region)
pat gene expression cassette	
35S PRO	35S constitutive promoter [1)] derived from cauliflower mosaic virus (CaMV)
pat	A gene that encodes phosphinothricin acetyltransferase (PAT protein), derived from *S. viridochromogenes*
35S TERM	35S terminator to terminate transcription from cauliflower mosaic virus (CaMV)

[1)] Constitutive promoter: A promoter that drives the expression of target genes in all sites in plant body

Table 3 Origins and functions of the component elements of the donor nucleic acid used for the development of MIR604

Gene cassette resistant to Coleoptera	
Component elements	Origin and function
MTL	A promoter derived from the *metallothionein* gene of maize. Since Corn Rootworm, the target insect of the order Coleoptera, eats and damages the roots of maize, *MTL* promoter is used to define the start of transcription of target genes in the roots.
Modified *cry3Aa2*	A modified version of the *cry3Aa2* gene, which is derived from *B. thuringiensis* subsp. *tenebrionis*, a typical gram-positive soil microorganism forming spores, by modifying some nucleotide sequences to change the contents of GC and enhance its expression level in plants and transferring cathepsin G protease recognition sequence to enhance the activity against Corn Rootworm. This gene encodes the modified Cry3Aa2 protein.
Nos	The terminator region of the nopaline synthase gene of *A. tumefaciens*, which terminates transcription and induces polyadenylation.
Selective marker gene cassette	
Component elements	Origin and function
ZmUbiInt	A promoter derived from the *polyubiquitin* gene of maize, to define the start of transcription of target genes in the entire plant body of monocotyledon.
pmi	The gene derived from *Escherichia coli* (*E. coli*.), which encodes the PMI protein (phosphomannose isomerase). The PMI protein is an enzyme that has the capability of catalyzing the reversible interconversion of mannose-6-phosphate and fructose-6-phosphate. Transferring of this enzyme allows utilization of mannose as a carbon source. The *pmi* gene was used for selection of transformed cells.
Nos	The terminator region of nopaline synthase gene of *A. tumefaciens*, and terminates transcription and induces polyadenylation.
Other regions	
Component elements	Origin and function
Spec	The streptomycin adenylyltransferase gene *aadA*, derived from the transposon Tn7 of *Escherichia coli* (*E. coli*). This gene is used as a bacteria selective marker to confer the resistance to erythromycin, streptomycin and spectinomycin.
VS1 ori	The replication origin consensus sequence derived from the plasmid pVS1 of *Pseudomonas* bacteria. Functions as the replication origin of plasmid in *A. tumefaciens*.
ColE1 ori	The replication origin that permits replication of plasmid in bacteria.
LB	T-DNA left border region derived from *A. tumefaciens* nopaline Ti-plasmid.
RB	T-DNA right border region derived from *A. tumefaciens* nopaline Ti-plasmid.
VirG	A region involved in transfer of T-DNA, derived from *A. tumefaciens*.
RepA	The pVS1 replication protein derived from *Pseudomonas* bacteria, taking on part of the responsibility for replication of pVS1 in the gram-positive bacteria living parasitically in plants.

(All the rights pertinent to the information in the table above and the responsibility for the content rest upon Syngenta Japan K.K.)

Table 4 Origins and functions of the component elements of the donor nucleic acid used for the development of Cry1F line 1507

Gene cassette resistant to Lepidoptera	
Component elements	Origin and function
UBIZM1(2) Promoter	Ubiquitin constitutive promoter derived from *Zea mays* [1] (including intron and 5' untranslated region) (Reference 23).
cry1F	A gene that encodes Cry1F protein derived from *B. thuringiensis* var. *aizawai*. Optimized to activate the expression in plants (GenBank AAA22347).
ORF25PolyA Terminator	A terminator to terminate transcription from *A. tumefaciens* pTi5955 (Reference 24).
Gene cassettes tolerant to glufosinate herbicide	
Component elements	Origin and function
CAMV35S Promoter	35S constitutive promoter [1] derived from cauliflower mosaic virus (CaMV) (Reference 25).
Pat	A gene that encodes phosphinothricin acetyltransferase (PAT protein), derived from *S. viridochromogenes*. Optimized to activate the expression in plants (Reference 26).
CAMV35S Terminator	35S terminator to terminate transcription from cauliflower mosaic virus (CaMV) (Reference 25).

[1] Constitutive promoter: A promoter that drives the expression of target genes in all sites in plant body

Table 5 Origins and functions of the component elements of the donor nucleic acid used for the development of GA21

Gene cassette tolerant to glyphosate herbicide	
Component elements	Origin and function
Act promoter ＋intron	A promoter derived from the rice actin 1 gene inducing the initiation of transcription of target gene throughout the entire plant body, including up to the first intron region which functions to enhance the efficiency of transcription (Reference 27).
sssu＋mssu (Hereinafter referred to as "OTP")	The optimized transit peptide (OTP) sequences composed of the ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo) gene derived from chloroplast transit peptide sequence (sssu) from sunflowers and the *RuBisCo* gene derived chloroplast transit peptide sequence (mssu) from maize, functions to transport the mEPSPS protein expressed by the target gene *mEPSPS* gene to chloroplasts, where the protein takes action (Reference 28).

mEPSPS	A gene obtained from mutation of the 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS) maize gene (Reference 29); It encodes the 5-enol-pyruvyl-shikimate-3-phosphate synthase (mEPSPS), the activity of which is not inhibited by the glyphosate herbicide, with the 102nd amino acid threonine in the wild-type EPSPS amino acid sequence modified to isoleucine, and the 106th proline modified to serine (Reference 1).
NOS	A polyadenylation sequence of the nopaline synthase (NOS) gene from *A. tumefaciens*, terminating transcription (Reference 17).
Backbone region	
Component elements	Origin and function
amp	Consists of the lac sequence (Reference 22), composed of partial coding sequence for lacI derived from bacteriophage M13, promoter plac and partial coding sequence for β-galactosidase or lacZ protein, and the β-lactamase gene (*bla*) (Reference 30) conferring the ampicillin tolerance derived from plasmid pBR322 of *Escherichia coli* (*E. coli*); selects and maintains the *Escherichia coli* (*E. coli*) which contains the constitutive plasmid by expression of β-lactamase.
ori-puc	The replication origin region derived from the plasmid pUC19 of *Escherichia coli* (*E. coli*), conferring the autonomous replication potency of the plasmid in *Escherichia coli* (*E. coli*) (Reference 31).

2) Function of component elements

(a) Functions of individual component elements of donor nucleic acid, including target gene, expression regulatory region, localization signal, and selective marker

Functions of individual component elements of donor nucleic acid used for the development of Bt11, Event DAS-59122-7, MIR604, Cry 1F line 1507 and GA21 are individually shown in Table 1 to Table 5 (pp. 2 - 7).

(b) Functions of proteins produced by the expression of target genes and selective markers, and the fact, if applicable, that the produced protein is homologous with any protein which is known to possess any allergen (except allergenicity as food)

[The insecticidal protein]
The insecticidal proteins, isolated from the soil microorganism *B. thuringiensis*, exhibit their insecticidal activities against specific species of insects. It is indicated that the Bt protein, when fed and digested by sensitive species of insects, becomes an active polypeptide (= core protein) through specific digestion of protein, which specifically binds to the specific receptors on the surface of midgut of insects, causing cytoclasis or cell-destruction and leading to destructed digestive tracts and death of the

insects (Reference 32). This mechanism of action also holds for the Cry1Ab protein, the Cry34Ab1 protein, the Cry35Ab1 protein, the Cry3Aa2 protein and the Cry1F protein.

Modified Cry1Ab protein:

Regarding the insecticidal activity of the modified Cry1Ab protein which has the core protein consisting of the same amino acid sequence as Cry1Ab protein, detailed experimental results are listed in the database operated by the Canadian Government (Reference 33), showing that it exhibits insecticidal activity against European corn borer (*Ostrinia nubilalis*), Corn earworm (*Helicoverpa zea*), Fall armyworm (*Spodoptera frugiperda*) and other order Lepidopteran insects which are the major pest insects for maize cultivation. On the other hand, the Cry1Ab protein exhibits little to no insecticidal activity against any insects other than the order Lepidoptera.

Cry34Ab1 protein+Cry35Ab1 protein:

As a result of the test to examine the function of the Cry34Ab1 ptorein and the Cry35Ab1 protein, it has been suggested that the Cry34Ab1 protein has a pore-forming activity on phospholipid membranes, and the Cry35Ab1 protein expands pores and increases permeability of membranes (in-house data of Pioneer Hi-Bred International Inc.). In the *in vivo* test, it was confirmed that the Cry34Ab1 protein alone offers the insecticidal activity against Corn rootworm, though, in the presence of Cry35Ab1 protein, the Cry34Ab1 protein works in concert to exhibit approximately eight (8) times higher effect compared to when it works alone (Reference 34). The Cry35Ab1 protein alone does not exhibit any insecticidal activity against Corn rootworm. In order to examine any morphological changes in the midgut tissue based on the immunohistochemical method, the larvae of Corn rootworm were fed with the recombinant maize which produces the Cry34Ab1 protein and the Cry35Ab1 protein. As a result, the larvae fed with the non-recombinant maize exhibited no abnormality, though the larvae fed with the recombinant plant showed the phenomena implying cell death such as swollen and/or vacuolated midgut cells, and foamy and/or dissolved cell membranes. The results demonstrate that the Cry34Ab1 protein and the Cry35Ab1 protein destroy the midgut as the target organ, like other Bt proteins (Reference 35).

In general, Bt proteins are known to have an extremely specific insecticidal activity (Reference 36). In fact, in the test of feeding a mixture of the Cry34Ab1 protein and the Cry35Ab1 protein conducted to examine the insecticidal spectrum against the six (6) kinds of pest insects for the cultivation of maize in the US, it was found that the proteins show high specific insecticidal activity against limited pest insects (Reference 37). Among the six (6) kinds of pest insects tested, the larvae of two (2) kinds of

pest insects of the order Coleoptera, Northern corn rootworm (*Diabrotica barberi*) and Western corn rootworm (*Diabrotica virgifera virgifera*), exhibited especially high insecticidal activity. For Southern corn rootworm (*Diabrotica undecimpunctata howardi*), which belongs to the same family of Corn rootworm, both proteins exhibit lower insecticidal activity than against Northern corn rootworm and Western corn rootworm. For the adults of European corn borer, Corn earworm and Black cutworm, which are pest insects of the order Lepidoptera, and for Western corn rootworm, a pest insect of the order Coleoptera, no mortality was observed even at the maximum dose obtained in the test.

In order to investigate any effects on the non-target insects of the order Coleoptera other than Corn rootworm, a bioassay was conducted with two (2) species of ladybug (*Hippodamia convergens* and *Coleomegilla maculata*) (Reference 37). As a result of the bioassay, even at the maximum dose obtained in the test, no effect on the adults of *H. convergens* was observed. For the larvae of *C. maculata*, a decrease in live weight was observed, though no mortality was observed even at the maximum dose obtained in the test.

In addition to the insects of the order Coleoptera, tests were also conducted to the mammals (Reference 38, Reference 39, Reference 40), birds (Reference 41), fish (Reference 42), and Lepidoptera (Reference 37), Hymenoptera (Reference 37), Neuroptera (Reference 37), Collembola (Reference 37) and other insects. As a result, it was confirmed that the Cry34Ab1 protein and the Cry35Ab1 protein exhibit no toxicity against any of the non-target organisms tested.

Modified Cry3Aa2 protein:

The modified *cry3Aa2* gene has some nucleotide sequences modified to change the GC content for enhanced expression in the recipient organism of maize. In addition, in order to enhance insecticidal activity against Corn Rootworm, the target insect of order Coleoptera, the nucleotide sequence was modified as follows; the 108^{th} to 110^{th} amino acid sequence (valine-serine-serine) of the Cry3Aa2 protein was changed to be four (4) amino acids (alanine-alanine-proline-phenylalanine), the cathepsin G protease recognition sequence. This modification causes the modified Cry3Aa2 protein to be cut at the C-terminal side of phenylalanine, the 4^{th} amino acid of cathepsin G protease recognition sequence, and to become an active polypeptide (= core protein) in the midgut of Corn Rootworm. However, the amino acid sequences other than described above remain unchanged from those in the Cry3Aa2 protein derived from *Bacillus thuringiensis* subsp. *tenebrionis*.

The modified Cry3Aa2 protein exhibits insecticidal activity against the following four (4) Coleopteran insects [Western corn rootworm (*D. virgifera virgifera*), Northern corn rootworm (*Diabrotica longicornis barberi*), Colorado potato beetle (*Leptinotarsa decemlineata*) and Banded cucumber beetle (*Diabrotica balteata*)]. However, the modified Cry3Aa2 protein does not exhibit any insecticidal activity against Coleopteran insects including Southern corn rootworm (*Diabrotica undecimpunctata*) and Cotton ball weevil (*Anthonomus grandis*). In addition, the Cry3Aa2 protein exhibits little to no insecticidal activity against any insects other than the order Coleoptera.

Cry1F protein:

In order to investigate the insecticidal spectrum of the Cry1F protein, the Cry1F protein produced in the *Pseudomonas fluorescens* was added to artificial feeds, which were given to 15 different kinds of insects of the order Lepidoptera which are considered typical pest insects for the farming in the US. Among the 15 kinds of insects of the order Lepidoptera, six (6) are regarded as pest insects for maize grown in the US and the other nine (9) for cotton, soybean, canola and other crops. Among the six (6) maize pests, the Cry1F protein demonstrated greater insecticidal activity against European corn borer (*O. nubilalis*), Fall armyworm (*S. frugiperda*) and Beet armyworm (*Spodoptera exigua*), which are target insects of the Cry1F line 1507. With regard to the other three (3) maize pests, Southwestern corn borer (*Diatraea grandiosella*), Black cutworm (*Agrotis ipsilon*) and Ballworm, the Cry1F protein demonstrated lower insecticidal activity. Also for *Danaus plexippus*, which is not regarded as an agricultural pest insect, a test was conducted, though, even at the maximum dose obtained in the test, the death rate of *Danaus plexippus* was found equivalent to that in the control plot. Based on the results, it was found that the Cry1F protein has highly specific insecticidal spectrum similar as for other Bt proteins (Reference 36), and thus it offers insecticidal activity against limited insects.

In addition to the insects of the order Lepidoptera, tests were also conducted to the mammals, birds, fish, and Coleoptera, Hymenoptera, Neuroptera, Collembola and other insects. As a result, it was confirmed that the Cry1F protein exhibits no toxicity against any of the non-target organisms tested (Reference 43).

[Herbicide tolerant protein]

PAT protein:

Glufosinate inhibits glutamine synthase in plants causing plants to die due to accumulation of ammonia in the cells. However, the expression of the PAT protein acetylates and inactivates glufosinate, so that glutamine synthase in not inhibited.

mEPSPS protein:

The glyphosate herbicide, non-selective herbicide acting on stems and leaves, inhibits the activity of 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS), one of the enzymes in the shikimate pathway for aromatic amino acid biosynthesis, and interrupts the aromatic amino acid biosynthesis, thereby causing plants to die (Reference 44). The mEPSPS protein encoded by the *mEPSPS* gene exhibits EPSPS activity even in the presence of glyphosate herbicide, enabling aromatic amino acid biosynthesis, thereby conferring tolerance to glyphosate herbicide.

[Selective marker]

PAT protein:

Glufosinate inhibits glutamine synthase in plants causing plants to die due to accumulation of ammonia in the cells. However, the expression of the PAT protein acetylates and inactivates glufosinate, so that glutamine synthase in not inhibited.

PMI protein:

The *pmi* gene is derived from *Escherichia coli*, and encodes the PMI protein (phosphomannose isomerase). The PMI protein catalyzes the reversible interconversion of mannose-6-phosphate and fructose-6-phosphate. Generally, maize and many other plants cannot utilize mannose as a carbon source, though the cells containing the *pmi* gene can use mannose for their growth. For this reason, transferring the *pmi* gene into plant cells as a selective marker together with the target gene and subsequent incubation in the mannose-containing medium, transformed cells, including not only the *pmi* gene but also the target gene, can be selected (Reference 45). The PMI protein exists widely in nature, including the human digestive system and in fact, is present in soybean and other plants, though it has not been identified in maize.

A homology search using the publicly available database (FARRP, NCBI Entrez Protein database, etc.) indicated that the modified Cry1Ab protein, the Cry34Ab1 protein, the Cry35Ab1 protein, the modified Cry3Aa2 protein, the Cry1F protein, the PAT protein, the mEPSPS protein and the PMI protein do not share structurally related homologous sequences with any of the known allergens (modified Cry1Ab protein, 2010; Cry34Ab1 protein and Cry35Ab1 protein, 2003; modified Cry3Aa2 protein, 2010; Cry1F protein, 1999; PAT protein, 2010; mEPSPS protein, 2010; and PMI protein, 2010).

(c) Contents of any change caused to the metabolic system of recipient organism

There are non data suggesting that the modified Cry1Ab protein, the

Cry34Ab1 protein, the Cry35Ab1 protein, the modified Cry3Aa2 protein and the Cry1F protein possess enzymatic activity. Consequently, it is considered very unlikely that these proteins affect the metabolic pathway of maize of the recipient organism.

The PAT protein possesses very high substrate specificity to L-phosphinothricin (glufosinate herbicide) and dimethyl phosphinothricin, and there is no other protein or amino acid reported for the substrate of the PAT protein (Reference 46). Consequently, it is considered very unlikely that the PAT protein affects the metabolic pathway of maize of the recipient organism.

The mEPSPS protein is one of the enzymes that catalyze the shikimate pathway (Reference 47), and it is reported to react specifically with phosphoenolpyruvate (PEP) and shikimate-3-phosphate (S3P) (Reference 48). Consequently, it is considered very unlikely that the mEPSPS protein affects the metabolic pathway of maize of the recipient organism.

The PMI protein has the capability of catalyzing the reversible interconversion of mannose-6-phosphate and fructose-6-phosphate. The PMI protein reacts specifically with mannose-6-phosphate and fructose-6-phosphate, and there is no other natural substrate known for the PMI protein (Reference 49). Consequently, it is considered very unlikely that the PMI protein affects the metabolic pathway of maize of the recipient organism.

(2) Information concerning vectors

1) Name and origin

The plasmid vectors used for the development of Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21 are as follows.

Bt11: pZO1502 constructed based on the pUC18 derived from *Escherichia coli* (*E. coli*)
Event DAS-59122-7: PHP17662 constructed based on the vector pSB1 derived from *A. tumefaciens*
MIR604: pZM26 constructed based on the pUC19 derived from *Escherichia coli (E. coli)*
Cry1F line 1507: PHP8999 constructed based on the pUC19 derived from *Escherichia coli (E. coli)*
GA21: pDPG434 constructed based on the pUC19 derived from *Escherichia coli (E. coli)*

2) Properties

(a) The numbers of base pairs and nucleotide sequence of vector

The total number of base pairs of the plasmids used for the development of Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21 are listed below, and the nucleotide sequences of these plasmids have been determined.

Bt11: A total of 7,240 bp of the plasmid pZO1502
Event DAS-59122-7: A total of 50,311 bp of the plasmid PHP17662
MIR604: A total of 13,811 bp of the plasmid pZM26
Cry1F line 1507: A total of 9,504 bp of the plasmid PHP8999
GA21: A total of 6,128 bp of the plasmid pDPG434 (Reference 1)

(b) Presence or absence of nucleotide sequence having specific functions, and the functions

The nucleotide sequence having specific functions included in plasmids and used for the development of Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21 refers to the following antibiotic resistant marker genes. These antibiotic resistant marker genes are not transferred in the recipient organism.

Bt11: amp^R gene, resistance to ampicillin
Event DAS-59122-7: *tet* gene, resistance to tetracycline; and *spc* gene, resistance to spectinomycin
MIR604: spec gene, resistance to streptomycin, erythromycin and spectinomycin
Cry1F line 1507: *nptII* gene, resistance to kanamycin
GA21: amp^R gene, resistance to ampicillin (Reference 1)

(c) Presence or absence of infectivity of vector and, if present, the information concerning the host range

There are no data suggesting that the plasmids pZO1502, PHP17662, pZM26, PHP8999 and pDPG434 used for the development of Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21 contain any sequence showing infectivity.

(3) Method of preparing living modified organisms

1) Structure of the entire nucleic acid transferred in the recipient organism

Bt11:	A part obtained by cleaving the plasmid pZO1502 by the restriction enzyme *Not*I and removing the amp^R gene
Event DAS-59122-7:	Two (2) gene expression cassettes (insect pest-resistant gene cassette and herbicide glufosinate-tolerant gene cassette) transferred in the Event DAS-59122-7
MIR604:	Two (2) gene expression cassettes (insect pest-resistant gene cassette and selective marker gene cassette) between RB and LB of T-DNA region
Cry1F line 1507:	Two (2) gene expression cassettes (insect pest-resistant gene cassette and herbicide glufosinate-tolerant gene cassette) transferred in the Cry1F line 1507
GA21:	A DNA fragment composed of the herbicide resistant gene cassette (Act promoter + intron/OTP/*mEPSPS*/NOS) obtained by cleaving the plasmid pDPG434 by the restriction enzyme *Not*I (Reference 1)

2) Method of transferring nucleic acid transferred to the recipient organism

The following methods were used to transfer the nucleic acid to the recipient organisms.

Bt11: Electroporation method
Event DAS-59122-7: *Agrobacterium* method
MIR604: *Agrobacterium* method
Cry1F line 1507: Particle gun bombardment
GA21: Particle gun bombardment (Reference 1)

3) Processes of rearing of living modified organisms

(a) Mode of selecting the cells containing the transferred nucleic acid

Transformed cells were selected on the medium containing the substances listed below for individual recipient organisms.

Bt11: Glufosinate
Event DAS-59122-7: Glufosinate
MIR604: Mannose
Cry1F line 1507: Glufosinate
GA21: Glyphosate (Reference 1)

(b) Presence or absence of remaining *Agrobacterium* in case of using *Agrobacterium* method for transferring nucleic acid

Absence of remaining *Agrobacterium* in Event DAS-59122-7 was confirmed by transferring Event DAS-59122-7 to the carbenicillin-free medium and then observing the plant body under a microscope. In addition, for MIR604, after transferring of genes, the antibiotic Cefotaxime was added to the culture cell medium to remove any residual *Agrobacterium* used for the transformation. Then the PCR was carried out for regenerated plants, and the individual plants not containing the antibiotic-resistant marker gene in the backbone of plasmid were selected. Consequently, it is considered that there is no remaining *Agrobacterium.*

(c) Processes of rearing and pedigree trees of the following lines; cells to which the nucleic acid was transferred, the line in which the state of existence of replication products of transferred nucleic acid was confirmed, the line subjected to isolated field tests; and the line used for collection of other necessary information for assessment of Adverse Effect on Biological Diversity

This stack maize line was developed by cross-breeding method with use of Bt11 (maize resistant to Lepidoptera and tolerant to glufosinate herbicide), Event DAS-59122-7 (maize resistant to Coleoptera and tolerant to glufosinate herbicide), MIR604 (maize resistant to Coleoptera), Cry1F line 1507 (maize resistant to Lepidoptera and tolerant to glufosinate herbicide) and GA21 (maize tolerant to glyphosate herbicide). The status of approvals and applications for approval of Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21 in Japan are listed in Table 6 (p. 16).

Table 6 Status of approvals and applications for approval of Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21 in Japan

Lines	Safety as food	Safety as feed	Environmental safety
Bt11	March, 2001: Approved safety of use as food	March, 2003: Approved safety of use as feed	April, 2007: Approved for Type I Use Regulation
Event DAS-59122-7	October, 2005: Approved safety of use as food	March, 2006: Approved safety of use as feed	April, 2006: Approved for Type I Use Regulation
MIR604	August, 2007: Approved safety of use as food	August, 2007: Approved safety of use as feed	August, 2007: Approved for Type I Use Regulation
Cry1F line 1507	July, 2002: Approved safety of use as food	March, 2003: Approved safety of use as feed	March, 2005: Approved for Type I Use Regulation
GA21	March, 2003: Approved safety of use as food	March, 2003: Approved safety of use as feed	November, 2005: Approved for Type I Use Regulation
This stack maize line	February, 2011: Approved safety of use as food	November, 2010: Approved safety of use as feed	January, 2011: Pending application

(4) State of existence of nucleic acid transferred in cells and stability of expression of traits caused by the nucleic acid

1) Place where the replication product of transferred nucleic acid exists

It was confirmed that the transferred genes in Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21 exist on the maize genome.

2) The number of copies of replication products of transferred nucleic acid and stability of its inheritance through multiple generations

In Bt11, Event DAS-59122-7, MIR604 and Cry1F line 1507, as a result of Southern blotting analysis for the number of copies of the transferred gene, it was confirmed that one copy of each exists in the chromosome and that the transferred genes are all inherited stably through multiple generations.

In GA21, as a result of Southern blotting analysis for the number of copies of the transferred gene, it was confirmed that transferred genes exist in the chromosome at one site, it consists of six (6) consecutive regions derived from the fragment of the transferred herbicide-tolerant gene cassette (Act promoter + intron/OTP/*mEPSPS*/NOS), and that the transferred genes are all stably inherited through multiple generations.

3) The position relationship in the case of multiple copies existing in chromosome

—

4) Inter-individual or inter-generational expression stability under a natural environment with respect to the characteristics referred to specifically in (6)-1)

The stability of expression was identified as follows.

Bt11: Confirming the expression of proteins by ELISA method, the bioassay using pest insects of the order Lepidoptera, and glufosinate herbicide-spraying test

Event DAS-59122-7: Confirming the expression of proteins by ELISA method, the bioassay using pest insects of the order Coleoptera, and glufosinate herbicide-spraying test

MIR604: Confirming the expression of proteins by ELISA method, the bioassay using pest insects of the order Coleoptera

Cry1F line 1507: Confirming the expression of proteins by ELISA method, the bioassay using pest insects of the order Lepidoptera, and glufosinate herbicide-spraying test

GA21: Glyphosate herbicide-spraying test

5) Presence or absence, and if present, degree of transmission of nucleic acid transferred through virus infection and/or other routes to wild animals and wild plants

The transferred nucleic acid in Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21 do not contain any sequence allowing transmission. Therefore, it is considered unlikely that the nucleic acid transferred to those plants could be transmitted to any other wild animals and wild plants.

(5) Methods of detection and identification of living modified organisms and their sensitivity and reliability

For specific detection of the lines Bt11, MIR604 and GA21, a method based on the quantitative PCR analysis is available from the European Commission. The detection sensitivity is as follows in terms of the ratio of concentration of genome DNA: 0.08% and over for Bt11, 0.09% and over for MIR604 and 0.04% and over for GA21 (Reference 50, Reference 51). For the detection and identification of Cry1F line 1507, a quantitative analysis kit is available from GeneScan Europe AG (Freiberg, Germany) applying an RT (Real Time)-PCR method using the nucleotide sequence specific to Cry1F line 1507 as primers. For detection and identification of Event DAS-59122-7, a quantitative ELISA method using the polyclonal antibodies respectively for the Cry34Ab1 protein, the Cry35Ab1 protein and the PAT protein has been developed.

In order to detect and identify this stack maize line, the above-mentioned methods must be applied to each grain of maize seeds.

(6) Difference from the recipient organism or the species to which the recipient organism belongs

1) Specific contents of physiological or ecological characteristics that were accompanied by the expression of replication products of transferred nucleic acid

This stack maize line is given the traits as described below.

From Bt11: Resistance to Lepidoptera and tolerance to glufosinate herbicide due to the modified Cry1Ab protein and the PAT protein respectively which are derived from the transferred genes

From Event DAS-59122-7: Resistance to Coleoptera due to the Cry34Ab1 protein and the Cry35Ab1 protein and tolerance to glufosinate herbicide due to the PAT protein which are derived from the transferred genes

From MIR604: Resistance to Coleoptera and being a selective marker due to the modified Cry3Aa2 protein and the PMI protein respectively which are

derived from the transferred genes

From Cry1F line 1507: Resistance to Lepidoptera and tolerance to glufosinate herbicide due to the Cry1F protein and the PAT protein respectively which are derived from the transferred genes

From GA21: Tolerance to glyphosate herbicide due to the mEPSPS protein which is derived from the transferred genes

It is known that the modified Cry1Ab protein, the Cry34Ab1 protein, the Cry35Ab1 protein, the modified Cry3Aa2 protein and the Cry1F protein bind to the specific receptors on the surface of midgut of insects when fed and digested by sensitive species of insects. It is considered that the specificity of the Bt protein might be governed by the structure of protein and then the protein would bind to different receptors in the midgut cell of pest insects. It was confirmed that the Cry34Ab1 protein alone offers the insecticidal activity against Corn rootworm, though, in the presence of the Cry35Ab1 protein, the Cry34Ab1 protein works in concert to exhibit approximately up to eight (8) times higher effect compared to when it works alone (Reference 34). The Cry35Ab1 protein alone does not exhibit any insecticidal activity against Corn rootworm. In addition, there is no report that the modified Cry1Ab protein, the Cry34Ab1 protein, the Cry35Ab1 protein, the modified Cry3Aa2 protein and the Cry1F protein possess any enzyme activity. Therefore, it is considered unlikely that these proteins would affect the metabolic system of their recipient organisms. Consequently, it is considered unlikely that expression of the modified Cry1Ab protein, the Cry34Ab1 protein, the Cry35Ab1 protein, the modified Cry3Aa2 protein and the Cry1F protein in this stack maize line would cause emergence of any sensitive species of insects. Furthermore, there are no data suggesting that any stack lines in which multiple insect pest-resistant proteins are expressed exhibit combined effect in terms of resistance to pest insects.

The PAT protein possesses very high substrate specificity to L-phosphinothricin (glufosinate herbicide) and dimethyl phosphinothricin, and there is no other protein or amino acid reported for the substrate of the PAT protein (Reference 46). The mEPSPS protein is one of the enzymes that catalyze the shikimate pathway (Reference 47), and it is reported to react specifically with phosphoenolpyruvate (PEP) and shikimate-3-phosphate (S3P) (Reference 48). In addition, the PMI protein is an enzyme protein that catalyzes the reversible interconversion between mannose-6-phosphate and fructose-6-phosphate. The PMI protein reacts specifically with mannose-6-phosphate and fructose-6-phosphate, and there is no other natural substrate known for the PMI protein (Reference 49). In addition, the PAT protein, the mEPSPS protein and the PMI protein differ from each other with regard to their substrates, mechanism of action, and metabolic pathways. Consequently, it is considered unlikely that the PAT protein, the mEPSPS protein and the PMI protein would affect the metabolic system of their recipient organisms.

As mentioned above, based on the findings that the modified Cry1Ab protein, the Cry34Ab1 protein and the Cry35Ab1 protein, the modified Cry3Aa2 protein and the Cry1F protein expressed in this stack maize line differ from each other in the specificity, that they have been reported not to possess any enzyme activity, that the PAT protein possesses extremely high substrate specificity, that the mEPSPS protein reacts specifically to the phosphoenolpyruvic acid (PEP) and shikimate-3-phosphate (S3P), and that the PMI protein is specific to the mannose-6-phosphate and fructose-6-phosphate, it is considered unlikely that these proteins expressed in this stack maize line would interact with each other, change the metabolic system of their recipient organisms, and produce unexpected metabolites. Therefore, it is considered unlikely that these proteins would exhibit functional interaction.

In order to confirm that the proteins expressed in this stack maize line from the individual parent lines would interact with each other, this stack maize line was tested as below. As the non-recombinant control maize, the maize, which has the same genetic background (NP2222×5XH751) as this stack maize line used in the test, was subjected to tests.

[Bioassay using insects of the order Lepidoptera]

For investigating the resistance to Lepidoptera, this stack maize line, Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507, and their non-recombinant control maize were cultivated in three (3) fields in the U.S. (Shirley, Illinois; Slater, Iowa; and Wapela, Illinois). The European corn borer, the target pest insect, were inoculated at the 6th to 8th leaf stage of maize, and on the 14th to 20th day after inoculation, the severity of insect damage was observed visually.

As a result of the investigation, no significant difference was observed between this stack maize line, Bt11 and Cry1F line 1507 (Table 7, p.21). Therefore, it is considered that the insecticidal activity of this stack maize line against pest insects of the order Lepidoptera (European corn borer) virtually remains unchanged by crossing of parent lines.

Table 7 Severity of damage to plant body by pest insects of the order Lepidoptera (European corn borer) based on bioassay of this stack maize line

Location of test	This stack maize line		Bt11		Event DAS-59122-7		MIR604		Cry1F line 1507		Non-recombinant control maize	
	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation
Shirley, Illinois	1.0 b[1]	0.1	1.0 b	0.0	4.9 a	0.8	4.9 a	0.8	1.1 b	0.1	5.3 a	0.4
Slater, Iowa	1.0 b	0.0	1.0 b	0.0	4.3 a	0.4	4.2 a	0.8	1.1 b	0.1	4.8 a	0.3
Wapela, Illinois	1.0 b	0.0	1.0 b	0.0	6.9 a	0.1	6.8 a	0.2	1.0 b	0.0	6.9 a	0.3

Investigation for severity of insect damage was conducted for 10 plant bodies and 3 repeats.
1: Severity of insect damage was evaluated based on 9-step scales [1 (No damage) to 9 (Seriously damaged)]. (Reference 52)
2: Statistical treatment was conducted at each test site, and there is no significant difference in the mean value expressed in the same alphabetical letter (LSD after F-test, $p<0.05$).

[Bioassay using insects of the order Coleoptera]

Regarding resistance to Coleoptera, this stack maize line, Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and their non-recombinantmaize were cultivated in three (3) field in the U.S. (Bloomington, Illinois; Shirley, Illinois; and Tremont, Illinois), and the severity of insect damage of root by Western Corn Rootworm, the target pest insect was investigated. In the fields, where eggs of Western Corn Rootworm exist in soil, maize was cultivated to hatch eggs at the time of 2nd to 4th leaf stage of maize, and the severity of insect damage of root was observed visually at the time of silking.

As a result of investigation, in the field of Bloomington, Illinois, the severity of insect damage to this stack maize line was found significantly lower compared to Event DAS-59122-7. In this stack maize line, the protein resistant to the order Coleoptera from each of Event DAS-59122-7 and MIR604 was expressed. Therefore, it was considered that the amount of Coleopteran insect resistant protein in this stack maize line increased compared to the parent lines, and that combined effect was observed. As a result of investigation, in the fields in Shirley and Tremont, Illinois, no significant difference was observed, but the consistent trend as observed in the field in Bloomington, Illinois was identified between this stack maize line, Event DAS-59122-7 and MIR604.

Table 8 Severity of damage to plant body by pest insects of the order Coleoptera (Western Corn Rootworm) based on bioassay of this stack maize line

Location of test	This stack maize line		Bt11		Event DAS-59122-7		MIR604		Cry1F line 1507		Non-recombinant control maize	
	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation
Bloomington, Illinois	0.22 c[1]	0.15	2.91 a	0.05	1.26 b	0.66	0.77 bc	0.29	2.94 a	0.03	2.87 a	0.10
Shirley, Illinois	0.13 b	0.04	2.45 a	0.56	0.48 b	0.24	0.68 b	0.40	1.79 a	0.58	2.25 a	0.35
Tremont, Illinois	0.05 c	0.01	2.04 a	0.69	0.07 c	0.03	0.11 c	0.06	1.84 a	0.43	1.02 b	0.22

Investigation for severity of insect damage was conducted for 6 plant bodies and 3 repeats.

1: Degree of root damage by Western Corn Rootworm were evaluated based on the 16 scales from 0.01 (no damage; or one or two minor damage on the surface) to 3.00 (three nodes of the root were all damaged). (Reference 53)

2: Statistical treatment was conducted at each test site, and there is no significant difference in the mean value expressed in the same alphabetical letter (LSD after F-test, $p<0.05$).

[Bioassay using glufosinate herbicide]

Regarding tolerance to glufosinate, this stack maize line, Bt11, Event DAS-59122-7, Cry1F line 1507 and the non-recombinant control maize were cultivated in a greenhouse in the U.S., and the severity of injury by spraying of herbicide was investigated. At the 3rd leaf stage (on the 12nd day of sowing) of maize, herbicide glufosinate (Product name: Ignite™) was sprayed. On the 22nd day after spraying herbicide glufosinate, the severity of injury was observed visually. Glufosinate was sprayed at the concentration of 467 g active ingredient (a.i.)/ha (normal dosage), 3,736 g a.i./ha (8-times higher dosage) and 7,472 g a.i./ha (16-times higher dosage).

As a result of the investigation, the severity of insect damage to this stack maize line was found significantly higher compared to Bt11, Event DAS-59122-7 and Cry1F line 1507 when glufosinate was sprayed at higher concentrations (Table 9, p. 23). It was considered that the PAT protein from each of Bt11, Event DAS-59122-7 and Cry1F line 1507 was expressed in this stack maize line, resulting in higher amount of the PAT protein compared to the parent lines leading to combined effect.

Table 9 Investigation result of the severity of injury by spraying of herbicide glufosinate to this stack maize line

Concentration of herbicide (g.a.i/ha)	Levels of herbicide injury (%)									
	This stack maize line		Bt11		Event DAS-59122-7		Cry1F line 1507		Non-recombinant control maize	
	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation
467	0.0 h[1]	0.0	0.0 h	0.0	0.0 h	0.0	0.0 h	0.0	53.7 b	12.8
3,736	2.3 g	1.5	6.2 de	3.4	3.3 fg	2.7	4.5 ef	2.8	100 a	0.0
7,472	3.9 fg	2.3	9.4 c	3.3	6.0 de	2.6	7.7 cd	2.8	100 a	0.0

Investigation for severity of herbicide injury was conducted for 10 plant bodies and 3 repeats.

1: For each maize line, a non-sprayed plot was prepared. The level of herbicide injury in the non-sprayed plot is set as 0% (intact) for comparison. Then the levels of herbicide injury were evaluated based on the scale from 0% (intact) to 100% (complete death) in the herbicide sprayed plots.

2: There is no significant difference in the mean value expressed in the same alphabetical letter (Student-Newman-Keuls test, $p<0.05$).

(All the rights pertinent to the information in the table above and the responsibility for the content rest upon Syngenta Japan K.K.)

[Bioassay using glyphosate herbicide]

Regarding tolerance to glyphosate, this stack maize line, GA21 and the non-recombinant control maize were cultivated in a greenhouse in the U.S., and the severity of injury by spraying of herbicide was investigated. At the 3rd leaf stage (12nd day after sowing), herbicide glyphosate (Product name: Touchdown Total^{TM}) was sprayed. On the 22nd day after spraying herbicide glyphosate, the severity of injury was observed visually. Glyphosate was sprayed at the concentration of 840 g acid equivalent (a.e.)/ha refers (normal dosage), 3,360 g a.e./ha (4-times higher dosage) and 6,720 g a.e./ha (8-times higher dosage).

As a result, no significant difference between this stack maize line and GA21 was observed in the severity of herbicide injury (Table 10, p. 24). Therefore, it was confirmed that the tolerance of this stack maize line to herbicide glyphosate remains unchanged by crossing of parent lines.

Table 10 Investigation result of the severity of injury by spraying of herbicide glyphosate to this stack maize line

Concentration of herbicide (g.a.e/ha)	Levels of herbicide injury (%)					
	This stack maize line		GA21		Non-recombinant control maize	
	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation
840	4.4 d [1]	4.3	3.2 d	5.0	100 a	0.0
3,360	23.2 c	8.4	26.7 c	7.6	100 a	0.0
6,720	34.2 b	7.8	35.3 b	10.5	100 a	0.0

Investigation for severity of herbicide injury was conducted for 10 plant bodies and 3 repeats.

1: For each maize line, a non-sprayed plot was prepared. The level of herbicide injury in the non-sprayed plot is set as 0% (intact) for comparison. Then the levels of herbicide injury were evaluated based on the scale from 0% (intact) to 100% (complete death) in the herbicide sprayed plots.

2: There is no significant difference in the mean value expressed in the same alphabetical letter (Student-Newman-Keuls test, $p<0.05$).

(All the rights pertinent to the information in the table above and the responsibility for the content rest upon Syngenta Japan K.K.)

Based on the above results, some investigation showed significant differences regarding resistance to pest insects and the tolerance to herbicides; however, it was considered the differences were caused by combined effect. Therefore, it was concluded that the individual proteins expressed in the relevant parental lines do not interact with each other and that the traits obtained from the transferred genes remain unchanged in this stack maize line.

Consequently, regarding the differences in physiological or ecological characteristics between this stack maize line and the taxonomic species to which the recipient organism

belongs, evaluation was conducted based on the results of individual examinations on the parent lines Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21.

2) With respect to the physiological or ecological characteristics listed below, presence or absence of difference between genetically modified agricultural products and the taxonomic species to which the recipient organism belongs, and the degree of difference, if present

(a) Morphological and growth characteristics

For the morphological and growth characteristics of Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507, GA21 and their non-recombinant control maize, an assessment was conducted for the items listed in Table 11 (p. 26) in an isolated field in Japan. In all items examined except culm length of Event DAS-59122-7 and germination rate and ear diameter of Cry1F line 1507, no significant difference was observed, or comparable results presented. For Event DAS-59122-7 and Cry1F line 1507, a significant difference was observed from the non-recombinant control maize, though no consistent trend was identified between the two (2) species examined (Annex 1, 2, 4, 5 and 6; Confidential: Not disclosed to unauthorized person).

Table 11 Investigation results of morphological and growth characteristics of Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21

	Bt11	Event DAS-59122-7	MIR604	Cry1F line 1507	GA21
Start of germination	—	—	—	—	○
Uniformity of germination	○	○	○	○	○
Germination rate	○	○	○	○	○
Time of tasseling	○	○	○	○	○
Time of silking	○	○	○	○	○
Time of flower initiation	○	○	—	—	○
Time of flower completion	○	○	—	—	○
Flowering period	○	○	—	—	—
Culm length	○	○	○	○	○
Plant shape	○	○	○	○	○
Tiller number	○	○	○	○	○
Height of ear	○	○	○	○	○
Maturation time	○	○	○	○	○
Number of ears (Total number of ears)	○	—	○	○	○
Number of productive ears	○	○	○	○	○
Ear length	○	○	○	○	○
Ear diameter	○	○	○	○	○
Row number per ear	○	○	○	○	○
Grain number per row	○	○	○	○	○
Grain color	○	○	○	○	○
100-kernel weight	○	○	○	○	○
Grain shape	○	○	○	○	○
Fresh weight of above-ground parts at the harvest time	○	○	○	○	—
Plant weight at the harvest time (Total plant weight)	—	—	—	—	○

○:Examined

-: Notexamined

5

(b) Cold-tolerance and heat-tolerance at the early stage of growth

Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21 withered or died due to the low temperatures at the early stage of growth similarly to their non-recombinant control maize (Annex 1, 2, 4, 5 and 6; Confidential: Not disclosed to unauthorized person).

(c) Wintering ability and summer survival of the mature plant

Maize is a summer type annual plant, and after ripening the matured plant body usually withers and dies out. In fact, there is no report that, after maturity, maize has further propagated by vegetative parts, set seeds again, or produced seeds. Actually, at the end of isolated field tests, withering had begun and death after ripening was observed.

(d) Fertility and size of the pollen

As a result of the observation under a microscope with stained pollen, no difference was observed in fertility (maturity of the pollen due to staining), shape and size of the pollen between Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507, GA21, and their non-recombinant control maize (Annex 1, 2, 4, 5 and 6; Confidential: Not disclosed to unauthorized person).

(e) Production, shedding habit, dormancy and germination rate of the seed

Regarding seed production, comparisons were conducted between Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507, GA21 and their non-recombinant control maize for the characteristics referring to the production of seeds. A significant difference was observed between Cry1F line 1507 and its non-recombinant control maize regarding ear diameter. For Cry1F line 1507, a significant difference was observed from its non-recombinant control maize, though no consistent trend was observed between the two (2) species examined (Annex 1, 2, 4, 5 and 6; Confidential: Not disclosed to unauthorized person).

Regarding shedding habit of the seed, maize seed never shed spontaneously, since they adhere to ears and the ears are covered with husks (Reference 3). As observed in the non-recombinant maize, the ears of Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21, of were found covered with husk at harvest time.

Regarding germination rate of harvested seeds, Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21 all showed comparable results to those

of their non-recombinant control maize (Annex 1, 2, 4, 5 and 6; Confidential: Not disclosed to unauthorized person). Consequently, it was considered unlikely that the dormancy of Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21 would differ significantly from that of their non-recombinant control maize.

(f) Crossability

Crossability test was not performed for Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21 since there is no report that any wild relatives that can be crossed with maize are growing voluntarily in Japan.

(g) Productivity of harmful substances

As a result of plow-in tests, succeeding crop tests and soil microflora tests conducted for Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21, no significant difference from their non-recombinant control maize was observed in all the tests conducted for all the parent lines but Cry1F line 1507. A significant difference was observed in the fresh weight of lettuce in the succeeding crop tests and plow-in tests for Cry1F line 1507 and its non-recombinant control maize. However, no consistent trend was observed between the two (2) species examined (Annex 1, 3, 4, 5 and 6; Confidential: Not disclosed to unauthorized person).

Assessment Result by the Committee for Review on the Biological Diversity Risk Assessment

Name of the Type of Living Modified Organism: Maize resistant to Lepidoptera and Coleoptera, and tolerant to glufosinate herbicide and glyphosate herbicide **(**modified *cry1Ab*, *cry34Ab1*, *cry35Ab1*, modified *cry3Aa2*, *cry1F*, *pat*, *mEPSPS*, *Zea mays* subsp. *mays* (L.) Iltis) (Bt11×*B.t.* Cry34/35Ab1 Event DAS-59122-7 × MIR604 × *B.t.* Cry1F maize line 1507 × GA21, OECD UI: SYN-BTØ11-1× DAS-59122-7 × SYN-IR6Ø4-5 × DAS-Ø15Ø7-1 × MON-ØØØ21-9) [including the progeny lines isolated from the maize lines, Bt11, *B.t.* Cry34/35Ab1 Event DAS-59122-7, MIR604, *B.t.* Cry1F maize line 1507 and GA21, that contains a combination of any of the transferred genes in the individual maize lines (except those already granted an approval regarding Type 1 Use Regulation)]

Content of the Type 1 Use of Living Modified Organism: Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them

Applicant: Syngenta Japan K.K.

1. Item-by-item assessment of Adverse Effect on Biological Diversity

This stack maize line was developed by crossing of maize resistant to Lepidoptera and tolerant to glufosinate herbicide (Bt11) to which the modified *cry1Ab* gene and the *pat* gene were transferred; maize resistant to Coleoptera and tolerant to glufosinate herbicide (Event DAS-59122-7) to which the *cry34Ab1* gene, the *cry35Ab1* gene and the *pat* gene were transferred; maize resistant to Coleoptera (MIR604) to which the modified *cry3Aa2* gene and the *pmi* gene were transferred; maize resistant to Lepidoptera and tolerant to glufosinate herbicide (Cry1F line 1507) to which the *cry1F* gene and the *pat* gene were transferred; and maize tolerant to glyphosate herbicide (GA21) to which the *mEPSPS* gene was transferred. These parent lines were individually judged at the Committee for Review on the Biological Diversity Risk Assessment as causing no Adverse Effect on Biological Diversity when used in line with Type 1 Use described in the application for this stack maize line.

It was considered that the specificity of the Bt protein might be governed by the structure of protein and then the protein would bind to different receptors in the midgut cell of pest insects. In addition, there is no report that in the stack lines granted approvals to date, the Bt protein has exhibited combined effect. Consequently, it was considered unlikely that the individual Bt proteins (the modified Cry1Ab protein, the Cry34Ab1 protein, the Cry35Ab1 protein, the modified Cry3Aa2 protein and the Cry1F protein) would interact with each other in this stack maize line and affect the specificity of the Bt proteins. Furthermore, the

PAT protein, the mEPSPS protein and the PMI protein differ from each other with regard to their substrates, mechanism of action, and their involved metabolic pathways. There is no report that the Bt protein possesses any enzyme activity. Therefore, it was considered unlikely that these proteins, even if expressed in this stack maize line, would interact with each other to affect the metabolic system of their recipient organisms and produce any unexpected metabolites.

In addition, the resistance to Lepidoptera and the tolerance to glyphosate herbicide in this stack maize line were found at similar levels as exhibited by the individual parent lines. Regarding the resistance to Coleoptera and the tolerance to glufosinate herbicide, combined effect was observed, which is considered as being associated with the amount of expressed protein in this stack maize line. Consequently, it is considered unlikely that the proteins expressed in this stack maize line from individual parent lines would cause functional interaction in the plant body of this stack maize line, and it is considered unlikely that notable changes in traits have occurred in this stack maize line except for the traits that it received from the parent lines.

(1) Competitiveness

Maize (*Zea mays* subsp. *mays* (L.) Iltis), the taxonomical species to which the recipient organism belongs, has been long used in Japan, though there is no report that it has become self-seeding in the natural environment in Japan.

As a result of investigation for various characteristics referring to competitiveness of Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21, the parent lines of this stack maize line, a significant difference was observed between this stack maize line and its non-recombinant maize in some items examined. However, the differences were judged not to be large enough to enhance the competitiveness of this stack maize line.

This stack maize line is given traits to be resistant to the insects of order Lepidoptera and Coleoptera. However, it is not generally considered that the insect damage by Lepidopteran and Coleopteran insects is a major cause in making maize difficult to grow in the natural environment in Japan. Consequently, it is considered unlikely that this trait causes maize to become self-seeding in the natural environment and enhance the competitiveness. In addition, this stack maize line is given traits to be tolerant to glufosinate and glyphosate herbicides; however, it is unlikely that glufosinate and glyphosate herbicides are sprayed in the natural environment in Japan. Consequently, it is considered that this trait does not increase the competitiveness of this stack maize line. Moreover, this stack maize line is also given traits to be able to use mannose as a carbon source. However, it is not considered that this stack maize line uses the mannose as a carbon source in the natural environment in Japan. Therefore, it is considered unlikely that this trait

enhances the competitiveness of this stack maize line.

Based on the above understanding, it was judged that the following conclusion made by the applicant is valid: Regarding this stack maize line and the progeny lines of stack maize line isolated from the parent lines of this stack maize line, Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21, that contains a combination of any of the transferred genes in the individual parent lines, there are no specific wild animals and wild plants that are possibly affected by this recombinant maize, and it would pose no risk of Adverse Effect on Biological Diversity that is attributable to competitiveness.

(2) Productivity of harmful substances

Maize (*Zea mays* subsp. *mays* (L.) Iltis), the taxonomical species to which the recipient organism belongs, has been long used in Japan, though it is not generally known that the maize produces any harmful substances that could affect wild animals and wild plants.

It has been confirmed that the modified Cry1Ab protein, the Cry34Ab1 protein, the Cry35Ab1 protein, the modified Cry3Aa2 protein, the Cry1F protein, the PAT protein, the mEPSPS protein and the PMI protein expressed in this stack maize line do not have any homology with any of the known allergens.

In addition, the modified Cry1Ab protein, the Cry34Ab1 protein, the Cry35Ab1 protein, the modified Cry3Aa2 protein, the Cry1F protein, the PAT protein, the mEPSPS protein and the PMI protein expressed in this stack maize line are considered unlikely to interact with each other, change the metabolic system of their recipient organisms, and produce unexpected metabolites. Therefore, it is considered unlikely that these proteins would cause production of any harmful substances in this stack maize line. In practice, as a result of plow-in tests, succeeding crop tests and soil microflora tests conducted to examine the ability of the parent lines of this stack maize line, Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21, to produce any harmful substances (the substances secreted from the roots which can affect other plants and microorganisms, the substances existing in the plant body which can affect other plants after dying), there was no difference from the non-recombinant control plants observed in all tests that suggests a possible increase in the productivity of harmful substances of the parent lines.

The modified Cry1Ab protein, the Cry34Ab1 protein, the Cry35Ab1 protein, the modified Cry3Aa2 protein and the Cry1F protein expressed in this stack maize line exhibit the insecticidal activity against the insects of the order Lepidoptera and Coleoptera. Therefore, the Lepidopteran and Coleopteran insects were specified as wild animals and wild plants that are possibly affected by this recombinant maize.

There is a concern about possible effects on the Lepidopteran and Coleopteran insects which could eat directly this stack maize line or eat pollens dispersed from this stack maize line together with dietary plants. However, it is considered unlikely that the Lepidopteran and Coleopteran insects inhabit locally near the fields for cultivation of this stack maize line; therefore, it is considered extremely low that they could be affected in the level of population.

Based on the above understanding, it was judged that the following conclusion made by the applicant is valid: This stack maize line and the progeny lines of stack maize line isolated from the parent lines of this stack maize line, Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21, that contains a combination of any of the transferred genes in the individual parent lines, would pose no risk of Adverse Effect on Biological Diversity that is attributable to the production of harmful substances.

(3) Crossability

In the Japanese natural environment, there are no wild plants which can cross with maize. Therefore, it was judged that the following conclusion made by the applicant is valid: Regarding this stack maize line and the progeny lines of stack maize line isolated from the parent lines of this stack maize line, Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21, that contains a combination of any of the transferred genes in the individual parent lines, there are no specific wild animals and wild plants that are possibly affected by this recombinant maize, and it would pose no risk of Adverse Effect on Biological Diversity that is attributable to crossability.

2. Conclusion based on the Biological Diversity Risk Assessment Report

Based on the above understanding, the Biological Diversity Risk Assessment Report concluded that there is no risk that the use of this stack maize line and the progeny lines of stack maize line isolated from the parent lines of this stack maize line, Bt11, Event DAS-59122-7, MIR604, Cry1F line 1507 and GA21, that contains a combination of any of the transferred genes in the individual parent lines, in accordance with Type 1 Use Regulation causes Adverse Effect on Biological Diversity in Japan. It was judged that the conclusion above made by the applicant is reasonable.